National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 927c

Bovine Serum Albumin (7 % Solution)
(Total Protein Standard)

This Standard Reference Material (SRM) is intended primarily for use in the standardization of procedures employed in clinical analyses for total serum protein, for critical evaluation of daily working standards used in these procedures, and as a reference standard for assays of total protein by colorimetric methods. This SRM is a solution (mass fraction 7 %) of known protein concentration and purity. It conforms to the specification for standardized protein solution approved by the National Committee for Clinical Laboratory Standards (NCCLS) [1]. It is suggested that the biuret method [2] be used to standardize laboratory-prepared protein solutions and "normal" serum pools. Such standardized "normal" sera could then be used to calibrate refractometers or other instruments for serum protein estimations. SRM 927c may also be used for other procedures, such as gel diffusion, amino acid analysis, electrophoresis, nitrogen assays, or other tests that require well-characterized protein for calibration or evaluation. A unit consists of 10 ampoules each containing 2.1 mL of solution.

Certified Protein Concentration and Uncertainty: The biuret reference method [2] and a liquid chromatography (LC) method were employed to determine protein concentration in SRM 927c using SRM 927b as an external standard.

Certified Protein Concentration: 71.57 g/L ± 0.74 g/L

The certified value is the equally weighted mean of results obtained from two chemically independent methods. The uncertainty in the certified concentration is calculated as $U = ku_c + B$. The quantity u_c is the combined standard uncertainty calculated according to the ISO Guide [3], where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-method variation from both methods and material inhomogeneity. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and a 95 % level of confidence. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means.

Reference Values and Uncertainties: Reference values are provided in Table 1 for additional properties including fill volume, pH, sodium and chloride concentrations, density, absorbances at various wavelengths, and molecular mass as determined using electrospray ionization mass spectrometry. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Expiration of Certification: The certification of this SRM is valid until **30 September 2008**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is contaminated or modified.

The overall direction and coordination of technical measurements leading to the certification were performed by M.J. Welch of the NIST Analytical Chemistry Division.

Willie E. May, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 14 January 2004
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John Rumble Jr, Chief
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Analyses were performed by D.M. Bunk, M.S. Epstein, S.L. Love, J.M. Smeller, and L.T. Sniegoski of the NIST Analytical Chemistry Division.

The statistical analysis of the data used for certification was performed by L.M. Gill of the NIST Statistical Engineering Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and P. Fagan of the NIST Measurement Services Division.

Information Value: A literature value for the optical absorbance of bovine serum albumin is given in Table 2. This is a noncertified value with no reported uncertainty as there is insufficient information to assess uncertainty. This information value is given to provide additional characterization of the material.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

NOTICE AND WARNING TO USERS

SRM 927c IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. The blood used in the preparation of SRM 927c Bovine Serum Albumin (7 % Solution) was collected from cattle sourced in the United States. Only blood from cattle/carcasses that have passed ante-mortem, as well as, post-mortem USDA-Food Safety Inspection Service (FSIS) inspection were used. There were no additives to the pooled serum prior to protein purification.

INSTRUCTIONS FOR USE

Storage: This SRM is supplied to the user in sealed ampoules. The SRM should be stored in a refrigerator at a temperature between 2 °C and 8 °C. The ampoules should not be frozen because of possible breakage of ampoules during the thawing process. Once an ampoule is opened, the solution should be used promptly. Any unused solution in opened ampoules should be discarded.

Inappropriate Uses: This SRM is not intended to be used as a standard for dye-binding tests, for checking precalibrated refractometers, for immunochemical methods or as an additive for bilirubin standardization.

SOURCE, PREPARATION, AND ANALYSIS

Source of Material: The bovine serum albumin solution was prepared by Bionostics[1], Inc., Acton, MA, under contract with Bioreclamation, Inc., Hicksville, NY. The bovine serum was produced for manufacture into products for pharmaceutical use by West Laboratories, Inc. at USDA EST. #245-J, Iowa Beef Packers, Inc., Joslin, IL, USA.

The bovine serum albumin solution for this SRM was brought to proper ionic strength with sodium chloride and to proper pH with sodium hydroxide. It was sterilized by membrane filtration and tested for sterility by approved methods [4].

Preparation of Dilutions: Protein solutions of lower concentration may be prepared by transferring the appropriate aliquot to a volumetric flask and diluting to volume. Diluents are not furnished with the SRM; however, an aqueous sodium chloride diluent, such as a solution having a concentration of 0.15 mol/L, may be used.

Analytical Methods: All analyses for the certified and reference values were performed at NIST. Two independent methods were used for the determination of the protein concentration. One method was the biuret method used for previous lots of this SRM [2]. The second method used reversed-phase liquid chromatography with UV detection (215 nm). A Zorbax 300SB-C8 column, 2.1 mm x 150 mm, held at 30 °C was used for the analysis. The mobile phase involved a gradient elution that started at 70 % solvent A (0.03 % trifluoroacetic acid in water) and 30 % solvent B (0.026 % trifluoroacetic acid in acetonitrile) and finished at 20 % solvent A and 80 % solvent B.

[1]Certain commercial materials and equipment are identified to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

The reference values for various properties determined for SRM 927c are given in Table 1. Absorbances were measured in accordance with requirements specified for a standard BSA solution [1] and were performed on a Varian Cary 219[1] spectrophotometer. Measurement of pH was performed using an Orion Model 501 pH meter with a glass body combination pH electrode calibrated with pH 4 and pH 7 buffers. Sodium was determined using flame emission spectrometry with a stoichiometric air acetylene flame. Chloride was determined using a coulometric titration with electrogenerated Ag^+. Density was measured using the Lang-Levey pipet method [5]. Fill masses were determined gravimetrically and fill volumes were calculated from the fill masses and mean density.

Molecular mass was determined using LC/electrospray ionization mass spectrometry. The LC conditions were as those listed above. Measurements were performed on a Hewlett-Packard model 1100 LC/MSD operated in the positive ion mode. Two components were partially separated from one another and are in the ratio of approximately 3:2. The molecular masses of these components are shown in Table 1. The first component is in good agreement with the theoretical molecular mass based upon the amino acid sequence for BSA. The second component shows evidence of an unknown adduct. The previous lot (SRM 927b) also had two components with molecular masses similar to those measured for 927c.

Table 1. Reference Values for Various Properties of SRM 927c

Mean Fill Volume	2.181 mL	±	0.005 mL
pH	6.70	±	0.01
Sodium by Flame Emission Spectroscopy	29.4 mmol/L	±	0.4 mmol/L
Chloride by Coulometry	14.1 mmol/L	±	0.2 mmol/L
Density	1.0180 g/mL	±	0.0001 g/mL
Spectral Properties	Absorbance		
Ultraviolet (A_{252}/A_{279}ratio @ 1.0 g/L)	0.475	±	0.004
Soret Band (A_{405} @ 72 g/L)	0.135	±	0.003
Visible (A_{500} @ 72 g/L)	0.0259	±	0.0015
(A_{600} @ 72 g/L)	0.0109	±	0.0017
Molecular Mass	Daltons		
Primary Component	66 441	±	13
Secondary Component	66 561	±	35
Theoretical BSA from Amino Acid Sequence [6]	66 430		

The uncertainties in the reference values are calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO Guide [3], where u_c is intended to represent the measurement error at the level of one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and a 95 % level of confidence.

Table 2. Information Value for Optical Absorbance

Optical Absorbance @ 279 nm for 1 g/L [7]	0.667

REFERENCES

[1] Specification for Standardized Protein Solution (Bovine Serum Albumin), NCCLS Approved Standard: ASC-1, National Committee for Clinical Laboratory Standards, Villanova, PA, Second Edition, (1979).

[2] Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, T., Jr., and Schaffer, R., "A Reference Method for the Determination of Total Protein," Clin. Chem. **27** (10) pp. 1642-1654, (1981).

[3] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9 1st Ed. ISO, Geneva, Switzerland, (1993): see also Taylor, B.N., and Kuyatt, C.E., Guidelines for Evaluating and Expressing Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington DC, (1994); (available at http://physics.nist.gov/Pubs).

[4] United States Pharmacopeia, 21st Revision, p. 1350 Class A, United States Pharmacopeial Convention, Rockville, MD.

[5] Sniegoski, L.T. and Moody, J.R., "Determination of Serum and Blood Densities," Anal. Chem. **51**, pp. 1577-1578, (1979).

[6] Hirayama, K., Akashi, S., Furuya, M., and Fukuhara, K., "Rapid Confirmation and Revision of the Primary Structure of Bovine Serum Albumin by ESIMS and Frit-FAB LC/MS," Biochem. Biophys. Res. Commun. **173**, pp. 639-646, (1990).

[7] *All About Albumin-Biochemistry, Genetics, and Medical Applications*, Peters, T., Jr., Academic Press Inc., San Diego, CA, p. 25, (1995).

Certificate Revision History: 14 January 2004 (Editorial changes); 02 September 1999 (This technical revision reports the addition of an information value for optical absorbance); 11 June 1999 (This technical revision reports a change in the units for the certified protein concentration to g/L not mg/L); 10 May 1999 (original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: Telephone (301) 975-6776 (select "Certificates"), Fax (301) 926-4751, e-mail srminfo@nist.gov, or via the Internet http://ts.nist.gov/srm.